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Antibiotic Pre-exposure Reduces the Ability to Detect Heat Production of Bacteria in Biofilm

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Objectives

•To detect biofilm imbedded bacteria on glass beads by combined use of sonication and microcalorimetry, and
•To analyze the effect of antibiotic pre-exposure on the bacterial presence in biofilm.

Background

The microbiological diagnosis is crucial for treatment of **prosthetic joint infections (PJI)**. Antibiotic treatment before sampling of culture specimens might reduce the ability to culture bacteria resulting in misdiagnosis or inadequate treatment and outcome.
In this study we used **sonication[1]** and **isothermal microcalorimetry[2]** for the detection of biofilm bacteria.

Materials and methods

Materials: Biofilms of *Staphylococcus aureus* (ATCC 29213), *S. epidermidis* (ATCC 34985), *Escherichia coli* (ATCC 25922) and *Propionibacterium acnes* (ATCC 11827) were formed on porous glass beads according to a well-established protocol[3]. After 24 hour incubation (72 hours for *P. acnes*) of beads with bacteria, beads were individually exposed to vancomycin, rifampin, daptomycin, flucloxacillin (Staphylococci and *P. acnes*) or ciprofloxacin (*E. coli*) at increasing concentrations from 1 to 1024 times the minimal inhibitory concentration (MIC).

Methods: After overnight antibiotic exposure beads were sonicated to dislodge biofilm bacteria. The sonication fluid was investigated by microcalorimetry to detect bacterial presence by measuring growth-related heat flow (µW).
The lowest antibiotic concentration inhibiting heat production from biofilm-dislodged bacteria in 24 hour microcalorimetry (72 hours for *P. acnes*) was defined as the minimal heat inhibitory concentration (MHIC).
Quantitative culture methods were also performed in order to compare survival of planktonic bacteria to the amount of biofilm dislodged bacteria. All experiments were performed in triplicate.

Results

Vancomycin did not inhibit heat production of biofilm from staphylococci nor *P. acnes* at any concentration up to 1024 mg/l. Flucloxacillin inhibited *S. aureus* only at 128 mg/l (512x MIC), whereas with rifampin spontaneous resistance developed in biofilm bacteria at all concentrations up to 1024 x MIC (data not shown). Most effective in inhibiting heat production from biofilm was daptomycin inhibiting staphylococci and *P. acnes* at 128x, 64x and 32x MIC, respectively, as well as ciprofloxacin inhibiting *E. coli* already at 4x MIC.

Organism	Vancomycin		Daptomycin		Flucloxacillin		Ciprofloxacin	
	MIC	MHIC	MIC	MHIC	MIC	MHIC	MIC	MHIC
S. aureus	1	*>1024*	0.5	64	0.25	128	ND	ND
S. epidermidis	2	*>1024*	1	64	ND	ND	ND	ND
E. coli	ND	ND	ND	ND	ND	ND	0.015	0.0625
P. acnes	1	*>1024*	1	32	ND	ND	ND	ND

Table legend: Antibiotic- and species-specific measurements of minimal heat inhibitory concentration (MHIC, mg/l – see methods for details) compared to the minimal inhibitory concentration (MIC, mg/l) representing the lowest antibiotic concentration inhibiting growth of planktonic bacteria. ND: Not determined.

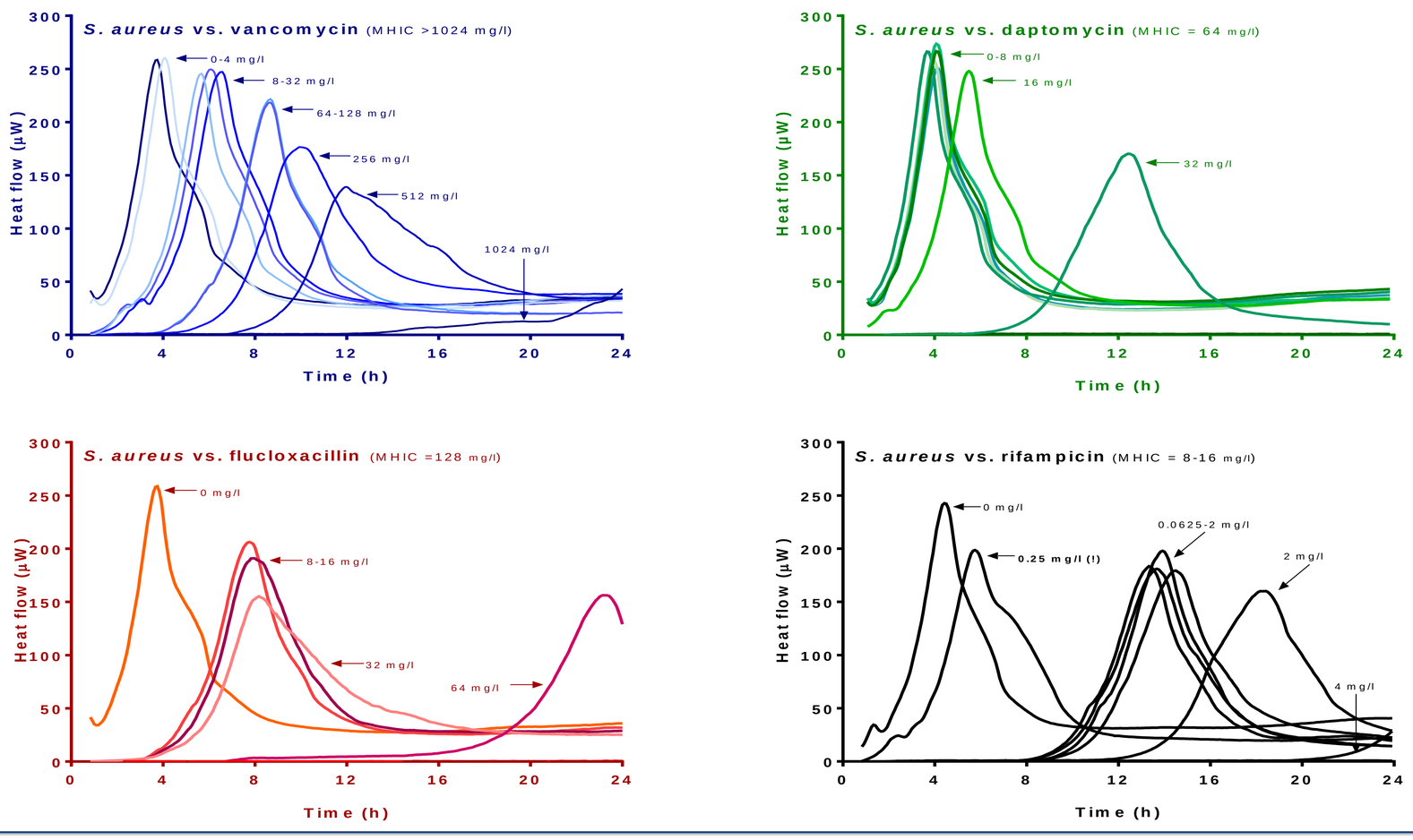

Figure legend: Heat flow curves of *S. aureus* after antibiotic exposure as a function of time. Each curve represents an antibiotic concentration. The time-shift of the curves depicts a delayed bacterial detection due to lower bacterial survival at increasing antibiotic concentrations. The numbers above the curve indicate the respective antibiotic concentrations. The number in bracket indicates minimal heat inhibitory concentration (MHIC).

Conclusion

These results indicate a higher ability for daptomycin and ciprofloxacin to penetrate biofilms compared to flucloxacillin and vancomycin commonly used in wound infections and PJI.

We found highly reproducible outcome and increased detection rate with the combined action of sonication and microcalorimetry in contrast to the basic culture methods. The findings of this study give encouraging perspectives for future biofilm investigation.

Reference list:[1]Trampuz A, 2007. NEJM; 357(7):654-63. [2]Bonkat G, 2012. BJU Int;110(6):892-7. [3]Furustrand Tafin U, 2012. AAC; 56(4):1885-91.
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